

Harold Varmus

TUMOR VIRUSES AND ONCOGENESI. Further Readings:

J.M. Bishop, The Molecular Biology of RNA Tumor Viruses: A Physician's Guide, New England Journal of Medicine 303:675-682, 1980, "Oncogenes", Scientific American 246:80, March 1982; Varmus, H.E., Form and Function of Retroviral Proviruses, Science 216:812-820, 1982; M. Weinberg, A Molecular Basis of Cancer, Sci. Amer., Nov. 1983; T. Krontiris, The Emerging Genetics of Human Cancer, NEJM 309:404-409, 1983. T. Hunter, The Proteins of Oncogenes, Sci. Amer. 251:70, Aug. 1984.

Marx, J.C. The Case of the Misplaced Gene. Science 218:983-985, 1982.

The Molecular Biology of Tumor Viruses, Part II, DNA Tumor Viruses, 1980; Part III, RNA Tumor Viruses, 1982, Cold Spring Harbor Laboratory.

Review of Medical Microbiology, chapter 40.

Davis, et al., Microbiology, Chapter 63.

Cold Spring Harbor Symposium on Quantitative Biology, Vol. 42, 1979.

Bunn et al., Clinical course of retrovirus-associated adult T-cell lymphoma in the U.S. New Engl. J. Med. 309:257-264, 1983.

Also see Tumor Virus Seminar and/or instructor for further readings.

II. Introduction

The evidence for a role for viruses in the etiology of most human tumors is sparse: epidemiological and biochemical studies suggest that conventional horizontally-transmitted infectious agents are not important in the majority of human cancers. The prominent exceptions to these statements -- evidence that hepatitis B virus is a causative factor in hepatoma, that a human retrovirus may cause a form of T cell leukemia and lymphoma, and that herpes viruses may play some role in a few human cancers -- will be considered in the herpes virus and hepatitis virus lectures and in the seminar on tumor viruses. *in addition, ... HPV's*

This lecture is intended primarily to acquaint you with the rapid advances now being made in the understanding of how viruses cause cancers in animals other than man and how tumor virology has introduced us to a class of cellular genes, called proto-oncogenes, that appear to be activated in many human tumors of unknown etiology. Whether or not human cancer is virus-related, animal tumor viruses offer an important opportunity to learn how cells work, how their behavior can be altered by a very small number of viral genes, and how cellular genes related to viral genes might be implicated in human cancer.

III. General ConsiderationsA. Transformation

Tumor viruses, by definition, have the capacity to produce tumors upon infection of appropriate animals. In practice, most work with tumor viruses is now conducted in cultured animal cells; in the appropriate cells, tumor viruses generally have the capacity to cause an in vitro analogue of tumor production, referred to as "neoplastic transformation" of cells. Transformation of cells by tumor viruses involves a stably inherited change in one or more properties of

cell behavior; most commonly, such change includes altered morphology (transformed cells look more like tumor cells than like normal cells) and freedom from normal growth restraints (transformed cells, in contrast to normal cells, will grow in random array to a very high density, without attachment to a solid surface, and at low serum concentration; they will form a "focus" of piled-up cells, permitting an assay for viral transformation; and they will usually produce tumors when injected into animals). In addition, various biochemical changes (alteration in properties of cell membranes, production of new cellular and viral proteins, reduction of cyclic AMP levels, etc.) may also accompany transformation. As discussed below, a major focus of interest in tumor virology is an understanding of how a single viral gene can produce such a profound effect upon cell behavior.

B. DNA and RNA tumor viruses

Tumor viruses, like other viruses, are classified according to the nature of their genomes. There are many specific examples of RNA tumor viruses which exhibit a variety of biological effects, but (fortunately for students) the biochemical and structural properties of these viruses all appear very similar. The DNA tumor viruses are more complex, since the major classes of DNA viruses (papova viruses, hepatitis B viruses, adenoviruses, herpesviruses, and pox viruses) all have members which are tumorigenic (see appendix A and Table 1).

TABLE 1

DNA TUMOR VIRUSES: GENERAL PROPERTIES

Three size classes:

- small (DNA size 3-5 kb)---papovaviruses (SV40, polyoma, papilloma viruses; other human and simian isolates); hepatitis B viruses (man, woodchuck)
- medium (DNA size 40 kb)---adenoviruses (from several animal species, e.g., chickens, monkeys, man)
- large (DNA size 100-150kb) -herpesviruses (from several animal species, e.g., frogs, chickens, monkeys, man); pox viruses (generally produce benign tumors; found in several species)

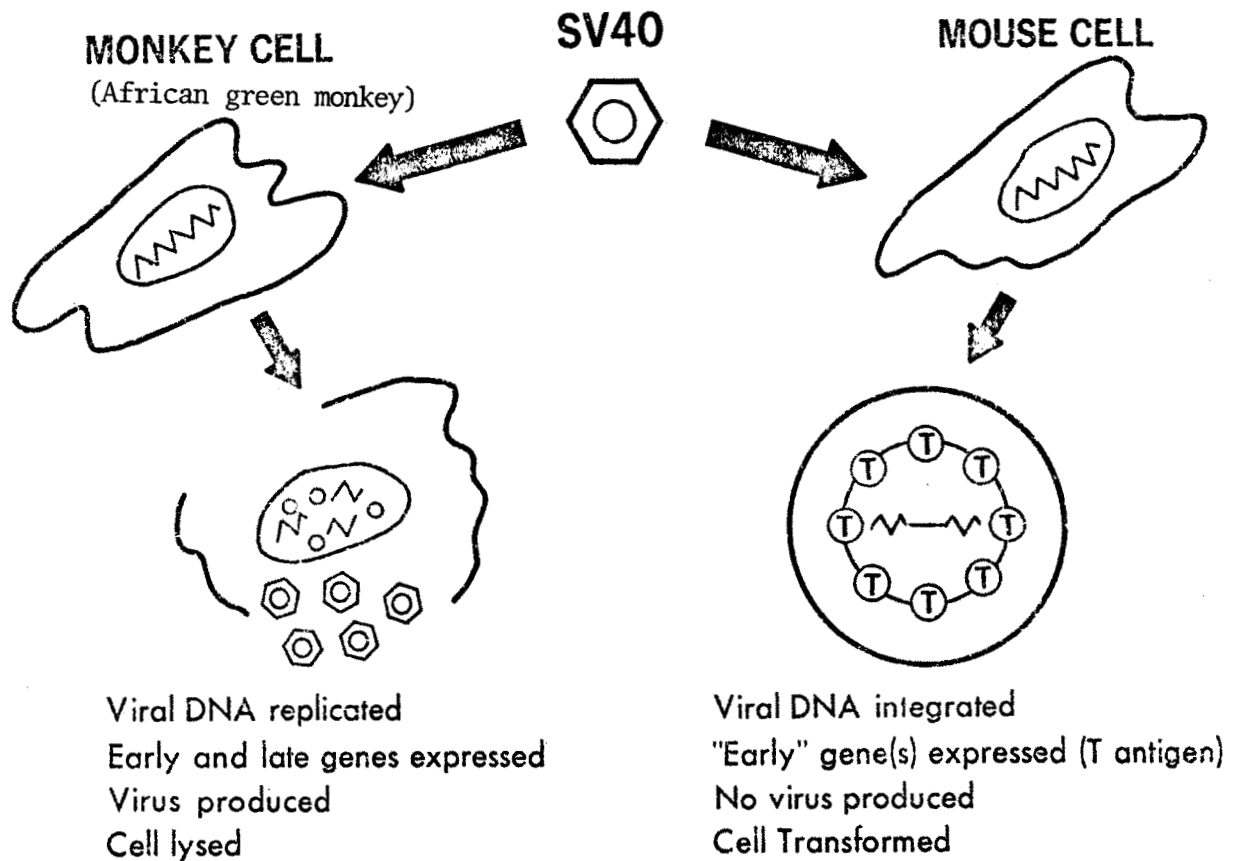
C. The lysogeny model

Since tumor viruses are able to produce stable alterations in host cells and since those changes appear to be maintained by the activity of viral genes, it is not surprising to learn that tumor virus DNA has the capacity to integrate covalently into the genome of the host cell; thus tumor viruses appear to be at least partially related to lysogenic bacteriophage (Table 2). As in the case of most bacteriophage, the genomes of many tumor viruses appear to pass through a stage in which they are in the form of double stranded DNA circles; this is even true of RNA tumor viruses (see below). There is no evidence that tumor viruses produce repressors of the sort responsible for the maintenance of lysogeny. However, in many cases, there is limited expression of viral genes in cells transformed by tumor viruses.

TABLE 2. RELEVANCE OF LYSOGENY MODEL TO TUMOR VIROLOGY

CLASS	EXAMPLE	GENOME	CIRCULAR dsDNA PHASE	INTEGRATION	VIRAL REPRESSOR	LIMITED EXPRESSION OF VIRAL GENES
Temperate phage	Lambda phage	Linear dsDNA	+	+	+	+
DNA tumor virus	SV40	Circular dsDNA	+	+	-	+
RNA tumor virus	Rous sarcoma virus	Linear ssRNA	+	+	-	In some cases

FIGURE 1. ALTERNATIVE CONSEQUENCES OF SV40 INFECTION IN DIFFERENT CELL TYPES



D. Permissive and nonpermissive cells

DNA tumor viruses, with the exception of some herpes viruses and perhaps hepatitis B viruses, do not normally produce tumors in their natural hosts, and cells from the natural host are generally permissive for virus replication. Ordinarily, DNA tumor viruses only transform cells when some component of the virus or the host cell is defective for replication. Most commonly, transformation by DNA tumor viruses is studied in foreign (or heterologous) host cells which, for generally unknown reasons, are defective in their ability to support viral replication (i.e., are nonpermissive for that virus). For example (Fig. 1) the DNA tumor virus SV40 (simian virus 40) replicates in monkey cells, produces cytopathic effects (e.g., vacuolization in rhesus monkey cells or lysis in African green monkey cells) but it transforms rodent cells (e.g., mouse, rat, hamster) in which it is unable to replicate. *papillomas*

RNA tumor viruses, by contrast, are frequently implicated in cancers in their natural hosts, and they are capable of transforming cells while replicating within them. However, RNA tumor viruses can also transform heterologous host cells in which they are unable to replicate. In other words, RNA tumor viruses can transform permissive or non-permissive host cells. *O, adenos*

IV. Properties of RNA Tumor Viruses (*Retroviruses*)

move to earlier section
RNA tumor viruses have several unusual characteristics which are not considered elsewhere in this course; these characteristics have profound implications for molecular biology and they have strongly influenced the search for human tumor viruses.

A. Structural and genetic features

RNA tumor viruses have been identified in a wide variety of animals (e.g., viper, fish, birds, rodents, ungulates, cats, and several primates including man) but their biochemical and structural features are highly similar:

1. The viruses are enveloped, leaving infected cells by budding through cytoplasmic membranes and entering cells by interacting with host receptors, as described for several virus classes in lectures on replication.

2. The genome is in the form of two identical subunits of 5-10 thousand bases of single stranded RNA.

3. The virus core contains a virus-coded RNA-directed DNA polymerase ("reverse transcriptase") that converts the RNA genome to double stranded DNA during the virus life cycle.

4. Newly synthesized viral DNA forms a closed circle and is then integrated into host chromosomes by a precise mechanism that generates a provirus structurally similar to many transposable elements (see previous lecture). The genes of the provirus are expressed by host cell machinery. Note that permissive cells are persistently infected and often transformed, *usually* *structural* *spontaneous* *lysed* by retroviruses.

5. Retroviruses require three genes for replication, but many viruses are defective for replication (due to deletions of one or more of these three genes)

regulatory *gag* *g* *pol* *env* *splice* *int.* *trans* *spec*

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and must be complemented by a helper virus that supplies the missing functions in co-infected cells (much as described for defective transducing phages in previous lectures). Many of these replication defective viruses carry a gene that mediates the oncogenic effects of the virus (a viral oncogene, derived from a normal cellular gene; see below). But retroviruses without oncogenes can also produce a variety of tumors and other kinds of pathology (e.g., anemia, osteopetrosis, etc.).

6. The most commonly studied RNA tumor viruses are:

avian sarcoma viruses (e.g., Rous sarcoma virus)
 avian leukosis virus
 mouse leukemia virus
 mouse sarcoma virus
 mouse mammary tumor virus
 feline leukemia and sarcoma viruses
 simian sarcoma virus
 human T cell lymphotropic viruses
human AIDS - lymphadenopathy virus

7. Retroviruses are able to infect germ line cells and retroviral proviruses are thus endogenous to the chromosomes of most if not all species, including man. Some properties of endogenous retroviruses are listed below:

- Genetically transmitted in the form of proviruses
- May be induced chemically (see Section VI.C below)
- Often xenotropic (grow well in foreign hosts, poorly in species from which they are isolated)
- Occasionally oncogenic (e.g., murine leukemia virus and murine mammary tumor virus) but more often not pathogenic
- Probably present in all vertebrates, including man

V. How do Viruses Transform Cells?

The major attraction of tumor viruses as laboratory tools is their capacity to alter cell behavior with very few genes. The dependence of transformation upon the continued synthesis of the products of these "viral oncogenes" has been shown by the use of temperature sensitive mutants with lesions in these genes (Table 3).

The genome of the most intensely studied DNA virus, SV-40, is divided into an "early" region (expressed prior to replication of viral DNA) and a "late" region. The "late" region consists of three overlapping genes for coat proteins, and it is not expressed in transformed cells (which do not produce virus, see above). The "early" region also ~~appears to contain~~ overlapping genes, encoding at least two proteins; genetic studies with viral mutants indicate that one or more of these "early" proteins are necessary for transformation of non-permissive cells and for replication of viral DNA in permissive cells.

The best studied RNA tumor virus, Rous sarcoma virus of chickens, has four genes, only one of which (the viral onc gene, called src in Rous sarcoma virus) mediates transformation. This gene, in contrast to the early region of SV40 DNA, is not required for virus replication; in addition, the src gene product transforms permissive as well as non-permissive cells. The product, a

phosphoprotein of 60,000 daltons, has the intriguing capacity to phosphorylate tyrosine residues in certain proteins and it is found mainly in plasma membranes. Furthermore, this gene has been derived from a normal cellular gene, called c-src (see below Section VII).

TABLE 3

EXAMPLES OF VIRAL ONCOGENES

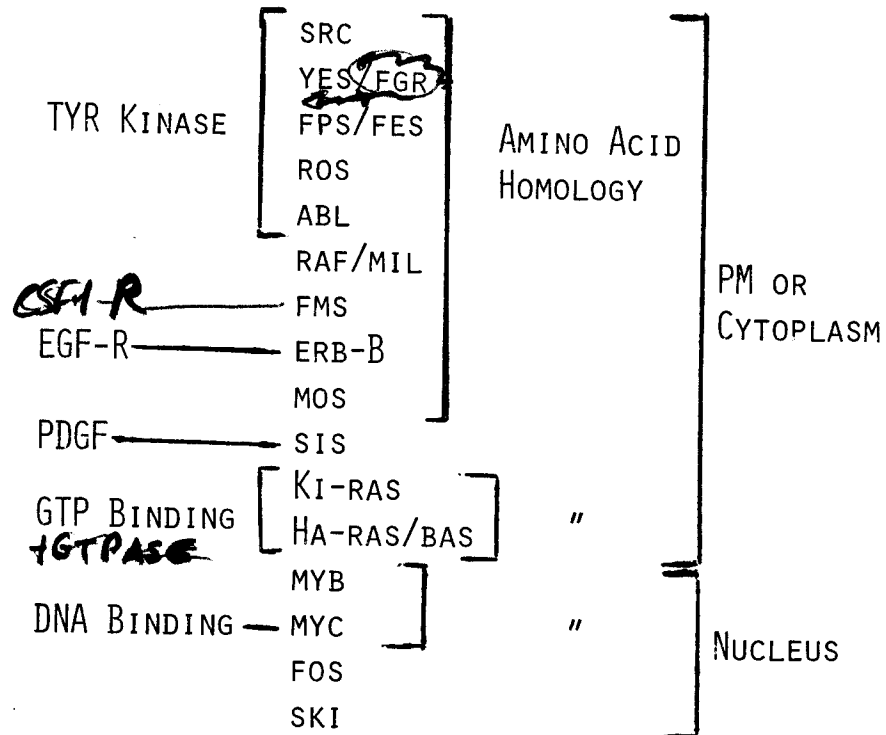
Property	Simian Virus 40	Rous Sarcoma Virus
Name	Early region (A gene)	<u>src</u> gene
Size of protein(s)	ca. 85,000 and 17,000	ca. 60,000
Required for viral replication	Yes	No
Required for trans- formation	Yes	Yes
Functions of product	DNA binding (stimu- lates DNA synthesis) ATPase (others)	Protein kinase (phos- phorylates tyrosine residues)
Location of product	Primarily nuclear	Plasma membrane (mainly)
Cellular homologue	No	Yes (see Section VII)

In addition to src, there are over 15 distinguishable retroviral oncogenes, each of which has been derived from normal cellular genes, called c-onc's or proto-oncogenes. Several of these other oncogenes also encode protein kinases specific for tyrosine and found at the plasma membrane, but most produce proteins that clearly have other kinds of biochemical properties (Table 4). In several cases, determination of the nucleotide sequences of the genes show that they are closely related, suggesting that retroviral oncogenes are derived from a few families of related cellular genes. Such genes are thought to function under normal circumstances as regulators of growth and development. In one case (sis) the gene has been shown to be derived from a host gene for a peptide hormone called platelet-derived growth factor (PDGF); in another case (erb B), the gene encodes the receptor for the epidermal growth factor (EGF). The importance of these proto-oncogenes in human cancer is discussed further below (Section VII).

CSF1 (R)

TABLE 4.

A SAMPLING OF RETROVIRAL ONCOGENES:
ANCESTRIES AND PROTEIN PRODUCTS



VI. Mechanisms of virus rescue

It is of inherent interest to know whether viral genes can be recovered from transformed cells which fail to produce virus (e.g., permissive cells transformed by defective viruses, or nonpermissive cells transformed by non-defective viruses). In addition, development of such techniques may be important in searches for human tumor viruses. Three types of approaches are generally used.

A. Cell fusion (Fig. 3). A transformed non-permissive cell is fused with an uninfected permissive cell to form a heterokaryon (cell with two or more different nuclei) which provides the factors necessary for virus replication. This method can be used with both RNA and DNA viruses.

RESCUE OF TUMOR VIRUS FROM NON-PERMISSIVE HOST

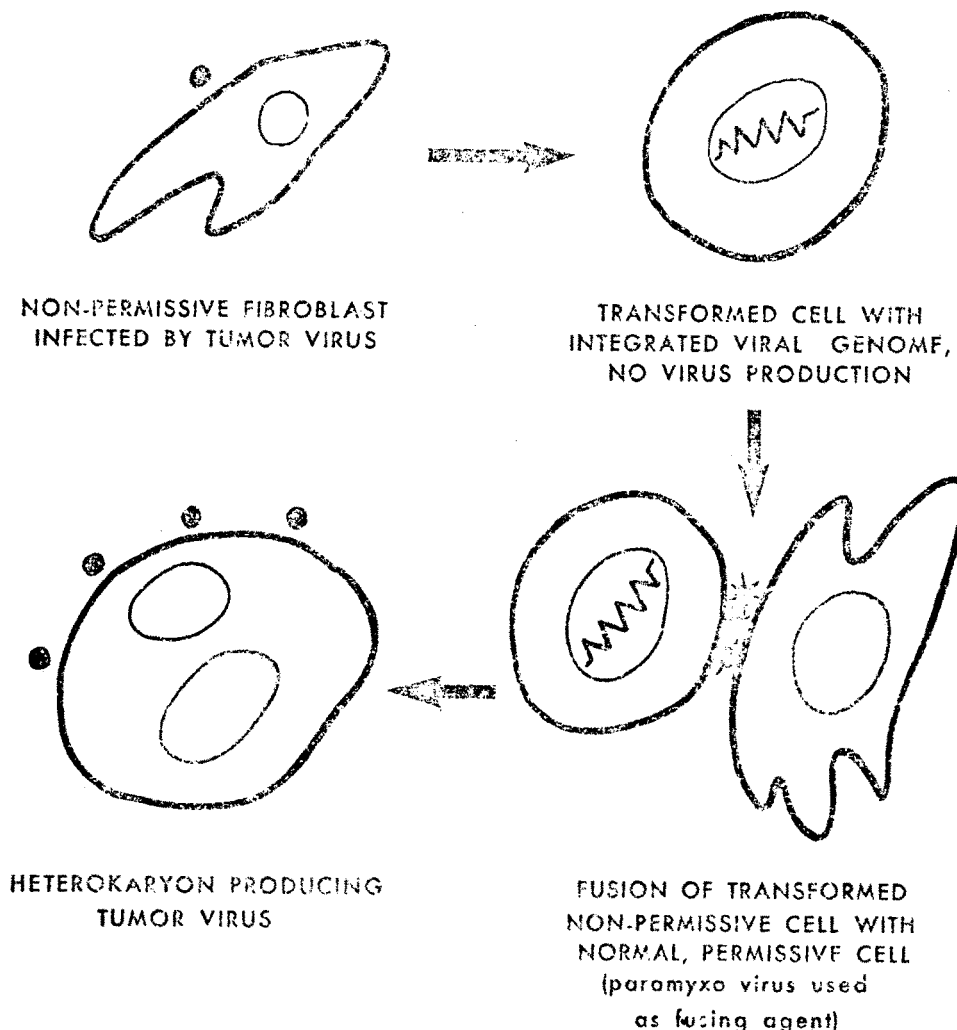


Figure 3

B. Helper virus. A permissive cell transformed by a virus defective in

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replication function(s) is superinfected by a "helper" virus with normal replicative powers. The helper virus provides the factors required for replication of the defective transforming virus, and both are produced by the cell. This phenomenon has been observed with both RNA and DNA viruses.

C. Chemical induction.

1) Treatment of certain non-permissive cells transformed by DNA tumor viruses with a variety of chemical agents (some of which induce lysogenic phage in bacteria) results in production of virus, presumably by facilitating excision of the integrated DNA genome.

2) Treatment of normal cells with similar inducing agents most commonly halogenated pyrimidines, such as iododeoxyuridine (IUdR) or bromodeoxyuridine (BUdR) often results in the production of endogenous RNA tumor viruses (see above Section IV). The mechanism of the induction is unknown. Only endogenous RNA viruses have been observed; their properties are considered more completely below. Production of retroviruses from exogenously-infected cells may also be augmented by such inducing agents (see Tumor Virus Seminar).

VII. Cellular proto-oncogenes

"Proto-oncogenes" in the genomes of normal cells were first discovered by molecular hybridization experiments performed with the viral gene (v-src) shown to be responsible for the oncogenic properties of Rous sarcoma virus (see part V, above). With each of over 15 different retroviral oncogenes the same extraordinary result has been obtained: a very similar gene is present in the normal genome of the animal species from which the virus was isolated. Moreover, these genes are not part of some endogenous retroviral provirus, but instead constitute genes recognized as cellular in origin by their architecture (introns and exons), their mode of expression, and their conservation throughout evolution. (All are present throughout vertebrates, including man, and some are even found in insects, worms, and yeast). Though the functions of most proto-oncogenes are not known, they generally produce proteins very similar to the protein products of viral oncogenes in small amounts, and it is presumed that their high degree of conservation bespeaks some fundamental role in growth or development (see Table 4). The appearance of these genes in the genomes of oncogenic RNA viruses implies that the genes were "transduced" by retroviruses that lacked oncogenes during their passage through host animals. The fact that each member of this group of genes can mediate oncogenic events (transformation of cultured cells and tumor induction in animals) when it is part of a viral genome suggests that each might have oncogenic potential in its natural setting, within the genomes of normal cells, e.g., if altered by mutations that affect the level of expression or the nature of the protein product (Fig. 4).

Several lines of experimentation support this view:

(1) In vitro manipulation. It has been shown that a few (though not all) cellular proto-oncogenes, e.g., c-ras and c-mos, directly isolated from normal cell genomes, can transform cells and make tumors, if programmed to be expressed efficiently when reintroduced into normal cells.

(2) Retroviral insertion mutations activate proto-oncogenes. Some of the

translocation of human

retroviruses that lack their own oncogenes (e.g., the chicken or mouse leukosis viruses or mouse mammary tumor virus) can nevertheless induce tumors of various types. Tumor production appears to be dependent upon the stimulation of efficient expression of a "cellular oncogene" by proviral DNA inserted nearby during infection. This mechanism has been clearly implicated in the case of B cell lymphomas in chickens, since proviral DNA is almost always found adjacent to a known proto-oncogene (first identified by its virtual identity to the viral oncogene in an acute leukemia virus of birds); in these tumors, the proto-oncogene, c-myc, is expressed about 50 times more efficiently than in normal B cells.

pure

(3) Point mutations of cellular ras genes make them oncogenic. DNA isolated from some tumors of animals and man, regardless of whether a causative agent (e.g., chemical or virus) is known, can transform the behavior of cultured mouse fibroblasts from a normal to malignant phenotype. The most likely interpretation of these results is that a mutation has occurred during the development of the original tumor, rendering a normal cellular proto-oncogene into a "transforming gene". Remarkably, several of these "transforming genes", including some isolated from human tumors of the lung, colon, and bladder, have proven to be members of the group of proto-oncogenes identified by their homology with viral oncogenes (Table 6). In several cases, the mutant "transforming" genes, all members of the ras gene family thus far (Table 4), have been cloned in bacteria and subjected to nucleotide sequencing. The results show that single base substitutions, different in each case but confined to two positions, codons 12 and 61 in the ras genes, are responsible for the altered properties of the cellular genes.

TABLE 6. Cellular Genes Implicated in Tumorigenesis
By the 3T3 Cell Transformation Assay

<u>Gene</u>	<u>Tumor</u>	<u>Alteration</u>
<u>c-Ha-ras</u>	Bladder CA Lung CA Mammary CA	Single base substitution
<u>c-Ki-ras</u>	Lung CA, Colon CA, others	Single base substitution
<u>N-ras</u>	Neuroblastoma Myeloid leukemia Rhabdomyosarcoma	Single base substitution

(4) Rearrangements of proto-oncogenes affects their expression and may render them oncogenic. In a number of human and murine tumors, the cellular homologues of retroviral oncogenes have been found to be grossly altered, either by (a) chromosomal translocations or (b) gene amplification (some examples are provided in Table 7.)

(a) The translocations frequently involve the same chromosomal sites in many tumors of the same type; most attention has been given to translocations in (human) Burkitt's lymphoma that join c-myc on chromosome 8, band q24, to the immunoglobulin heavy chain locus on chromosome 14, band q32, and translocations

Ph1 - bcr-abl

that join the same loci (on chromosomes 15 and 12) in mouse plasmacytomas. The translocations appear to alter the level of expression of c-myc in at least some cases, but the effect of the translocations upon the neoplastic process has yet to be defined.

(b) Amplification has been shown to occur in several types of tumors, affecting several oncogenes (Table 7). The amplified unit is large and can generally be seen during karyotyping as a homogeneously staining region within a chromosome or as multiple, small chromosomes lacking a centromere (double minute chromosomes). The increase in gene dosage is generally matched by an increment in oncogene expression. Amplification sometimes correlates with the staging of human cancers (e.g., N-myc in neuroblastomas) and may be useful in diagnosis.

TABLE 7.

CELLULAR GENES IMPLICATED IN NON-VIRAL
HUMAN AND MURINE TUMORS BY REARRANGEMENTS

<u>Gene</u>	<u>Tumor</u>	<u>Alteration</u>
c-myc	Burkitt's lymphoma Plasmacytoma	Translocations
c-abl	Myeloid leukemia	Translocation (Philadelphia chromosome)
c-myc	Myeloid leukemia Apudoma Small cell lung carcinoma	Amplification
c-Ki-ras	Adrenocortical Ca Colonial CA	Amplification
N-myc	Neuroblastomas Retinoblastomas <i>Small cell Ca lung</i>	Amplification

More than one of these varied mutations that affect cellular oncogenes are sometimes found within the same tumor cell. This is consistent with the generally accepted notion that full fledged cancer cells arise as a result of multiple events. A major goal of contemporary cancer research is the explicit definition of these several steps in the genesis of a cancer.

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The general implication of these several findings is that tumor viruses (in particular, retroviruses) may have led investigators to recognize a class of normal cellular genes ("cellular oncogenes") that may be involved in cancers initiated by various infectious, chemical, physical, or genetic mechanisms. Therefore, the normal functions of these genes, the kinds of changes they suffer during tumorigenesis, and the properties that make them oncogenic under certain circumstances are all matters of extraordinary interest to oncologists. As viewed in Table 8, these genes can be thought to have undergone a variety of "activating" events: transduction to become a viral oncogene, point mutation to produce a gene that transforms cultured cells; or insertion mutation, amplification, or translocation that affect gene expression. Lastly, in vitro,

some of these genes can be made oncogenic by addition of strong regulatory signals.

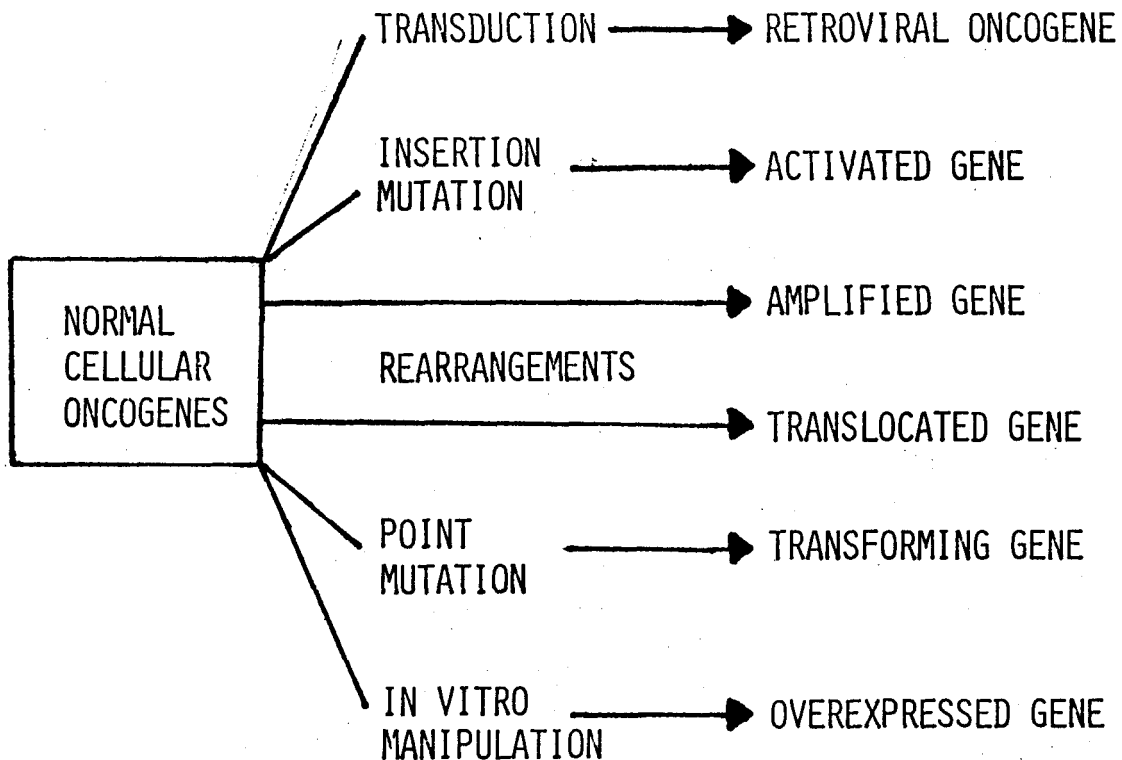


TABLE 8.

Appendix A. Additional Historical Information About DNA Tumor Viruses

Papova viruses ---viruses with small, circular (double-stranded) DNA genomes (ca. 5-8 kilobase pairs); "papova" derived from common members of this group: Papilloma viruses (from several natural hosts); Polyoma virus of mice; and Vacuolating viruses (causes vacuole formation upon infection of natural hosts) of which simian virus 40 (SV 40) is most famous example.

Papilloma viruses ---found in several species (including man) in which they replicate and cause warts (papillomata); grow poorly in cell culture and therefore not well studied. *low*

Polyoma virus ---common in wild mice in which the virus occasionally produces a variety of types of tumor (hence "poly" "oma," or many tumors); in cell culture, undergoes lytic, replicative cycle in its natural host (mouse) and transforms certain heterologous, nonpermissive hosts (e.g., hamster). *+ some - animal EA*

SV 40 ---discovered in rhesus monkey cells during development of polio vaccine; causes tumors in and transforms cells from certain heterologous, nonpermissive hosts (e.g., mouse and other rodents); undergoes replicative cycle in monkey cells.

Hepatitis B-type viruses ---discovered first in man (see hepatitis lectures), later in woodchucks, ground squirrels, and ducks; associated with hepatocellular carcinomas in man and woodchucks; inability to grow virus in tissue culture has slowed study of its replication and oncogenicity.

Adenoviruses ---viruses with medium-sized, linear (double-stranded) DNA genomes (ca. 35-40 kilobase pairs); found in many species, including man; the several human types show low, medium, or high oncogenicity when used to infect newborn rodents (especially hamsters) or rodent cells in culture (these are nonpermissive, heterologous hosts); replicate in cells from natural host (man) and cause mild, acute GI and respiratory illness (see lectures on respiratory infections).

Herpes viruses ---enveloped viruses with large, linear (double-stranded) DNA genome (ca. 150 kilobase pairs); associated with a variety of diseases, including tumors, in the several hosts in which these viruses have been found (e.g., chicken, frog, subhuman primates, and man); evidence for oncogenicity in man is not conclusive; virus replicates in cells from natural host, and, after irradiation to damage replication genes, it can transform certain heterologous host cells in culture (see herpes virus lecture).

Epstein-Barr virus (EBV) ---a herpes-like virus originally found in cells from African patient with Burkitt's lymphoma; now commonly seen in human lymphocytes from normal as well as diseased persons; it is the causative agent of infectious mononucleosis and has been indirectly implicated in causation of African Burkitt's lymphoma and nasopharyngeal carcinoma (see herpes virus lecture).

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FIGURE 4.

Handwritten: ~~normal~~ ~~cells~~

THE DOMINANT PARADIGM

NORMAL CELLS



CANCER CELLS

PROTO-ONCOGENES

MUTATION(S)
BASE CHANGES
REARRANGEMENTS

ONCOGENE(S)



NORMAL REGULATORS
OF GROWTH



INAPPROPRIATE AMOUNTS
OR ALTERED KINDS OF
REGULATORY PROTEINS